Studies of Key Quality Attributes for TIL Product, LN-144

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BACKGROUND

- Adoptive T-cell therapy with autologous tumor infiltrating lymphocytes (TIL) has demonstrated clinical efficacy in patients with metastatic melanoma and other tumors.[1-3]
- Most reports from clinical studies have included exploratory analyses of the infused TIL products with the intention of identifying quality attributes such as sterility, identity, purity, and potency that could relate to product efficacy and/or safety.[4,5]
- Here we present the evaluation of three key product parameters from Iovance TIL product LN-144 that may contribute to a future quality control platform for use in the commercial manufacture of TIL.

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1 2 3 4 5
Potency
Key critical quality attributes
Purity
Identity

Overview of TIL Therapy Process

1. The tumor is excised from the patient and transported to the GMP Manufacturing facility.
2. Upon arrival the tumor is fragmented and placed in flasks with IL-2 for a pre-Rapid Expansion Protocol (REP).
3. pre-REP TIL are further propagated in a REP protocol in the presence of irradiated PBMCs, anti-CD3 antibody (30 ng/mL), and IL-2 (3000 IU/mL).
4. TIL products are assessed for critical quality attributes including: 1) Identity 2) Purity, and 3) Potency.
5. Prior to infusion of expanded TIL (LN-144), patient receive a non-myeloablative lymphodepletion regimen consisting of cyclophosphamide and fludarabine. Following infusion of TIL, patients receive a short duration (up to 6 doses) of high-dose IL-2 (600,000 IU/kg) to support growth and engraftment of transferred TIL.

STUDY OBJECTIVES

- **Goal:** To fully characterize TIL products for identity, purity, and potency, and thereby
  - Guide the definition of critical quality attributes and
  - Support the establishment of formal release criteria to be implemented in commercial production of TIL products at Iovance.
- **Strategy:** To develop the following analytical methodologies to support TIL product characterization
  - Phenotypic analysis by flow cytometry for an identity and purity assessment
  - Residual tumor cell detection assay for a measure of purity
  - Interferon-gamma release assay for an assessment of potency

MATERIALS & METHODS

Identity + Purity

Phenotypic characterization: TIL products were stained with anti-CD45, anti-CD3, anti-CD8, anti-CD4, anti-CD45RA, anti CCR7, anti CD62L, anti-CD19, anti-CD16, and anti-CD56 antibodies and analyzed by flow cytometry for the quantification of T and non-T cell subsets.

Purity

Residual tumor detection assay: TIL products were stained with anti-MCSP (melanoma-associated chondroitin sulfate proteoglycan) and anti-CD45 antibodies, as well as a Live/Dead fixable Aqua dye, then analyzed by flow cytometry for the detection of melanoma cells. Spiked controls were used to assess accuracy of tumor detection and to establish gating criteria for data analysis.

Potency

IFNγ release assay: TIL products were re-stimulated with anti-CD3/CD28/CD137 coated beads for 18 to 24 hours after which supernatants were harvested for assessment of IFNγ secretion using an ELISA assay.

RESULTS

Identity: The majority (>99%) of melanoma TIL product is composed of $CD45^+CD3^+$

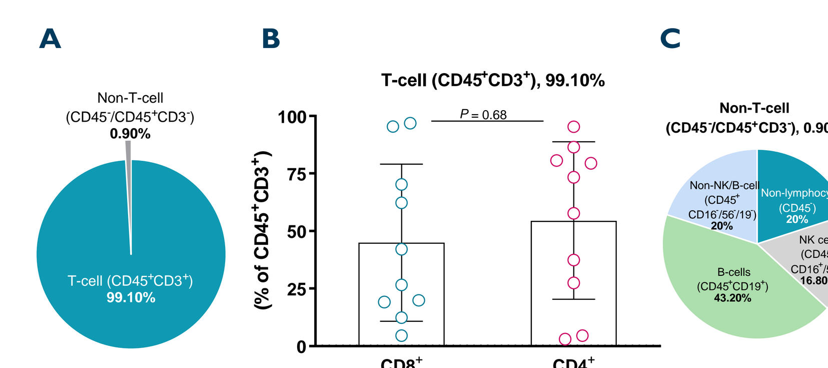


Figure 1. Phenotypic characterization of TIL products using 10-color flow cytometry assay. (**A**) Percentage of T-cell and non-T-cell subsets is defined by $CD45^+CD3^+$ and $CD45^-$(non-lymphocyte)/$CD45^+CD3^-$ (non-T-cell lymphocyte), respectively. Overall, >99% of the TIL products tested consisted of T-cell ($CD45^+CD3^+$). Shown is an average of TIL products (n=10). (**B**) Percentage of two T-cell subsets including $CD45^+CD3^+CD8^+$ (blue open circle) and $CD45^+CD3^+CD4^+$ (pink open circle). No statistical difference in percentage of both subsets is observed using student's unpaired T test ($P=0.68$). (**C**) Non-T-cell population was characterized for four different subsets including: 1) Non-lymphocyte (CD45-), 2) NK cell ($CD45^+CD3^-CD16^+/56^+$), 3) B-cell ($CD45^+CD19^+$) and 4) Non-NK/B-cell ($CD45^+CD3^-CD16^-CD56^-CD19^-$).

Identity: The majority of melanoma TIL product exhibits effector memory T-cell phenotype, associated with T-cell cytotoxic function

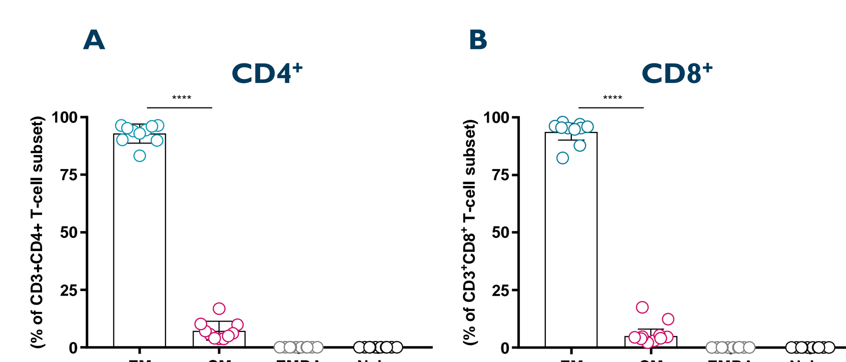


Figure 2. Characterization of T-cell subsets in $CD45^+CD3^+CD4^+$ and $CD45^+CD3^+CD8^+$ cell populations. Naïve, central memory (TCM), effector memory (TEF), and effector memory RA+(EMRA) T-cell subsets were defined using CD45RA and CCR7. Figures show representative T-cell subsets from 10 final TIL products in both $CD4^+$ (**A**), and $CD8^+$ (**B**) cell populations. Effector memory T-cell subset (blue open circle) is a major population (>93%) in both $CD4^+$ and $CD8^+$ subsets of TIL final product. Less than 7% of the TIL products cells is central memory subset (pink open circle). EMRA (gray open circle) and naïve (black open circle) subsets are barely detected in TIL product (<0.02%). p values represent the difference between EM and CM using student's unpaired T test.

Purity: MCSP represents an appropriate melanoma tumor marker for purity assay

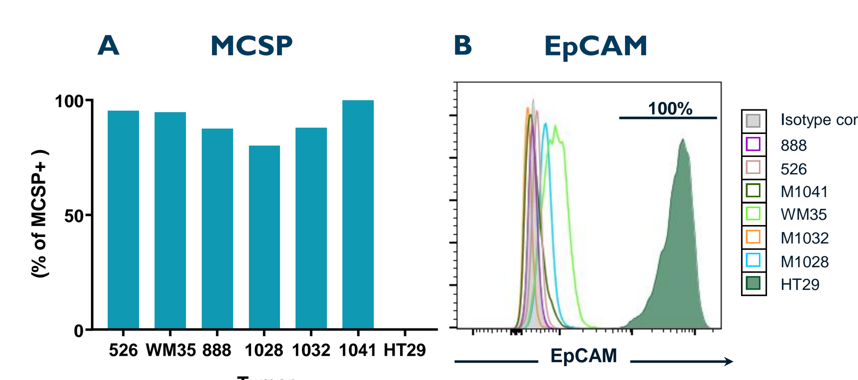
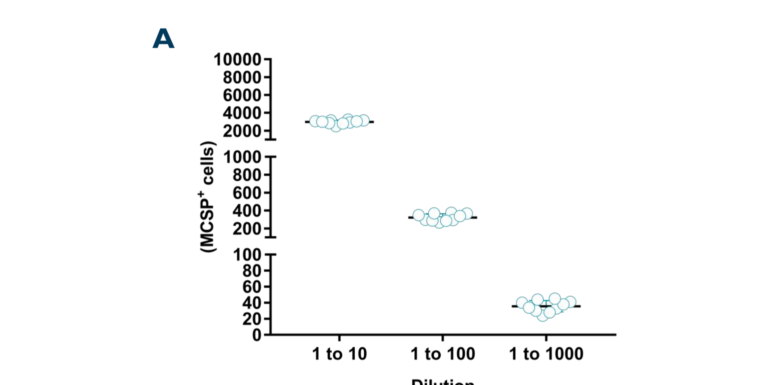


Figure 3. Detection of MCSP and EpCAM expression in melanoma tumor cells. Melanoma tumor cell lines (WM35, 526, and 888), patient-derived melanoma cell lines generated at Iovance Biotherapeutics (1028, 1032, and 1041), and a colorectal adenoma carcinoma cell line (HT29 as a negative control) were characterized by staining for MCSP (melanoma-associated chondroitin sulfate proteoglycan) and EpCAM (epithelial cell adhesion molecule) markers. (**A**) Average of 90% of melanoma tumor cells express MCSP. (**B**) EpCAM expression was not detected in melanoma tumor cell lines as compared positive control HT29, an $EpCAM^+$ tumor cell line.

Purity: Development of a flow cytometry-based assay for detection of residual tumor cells in TIL products

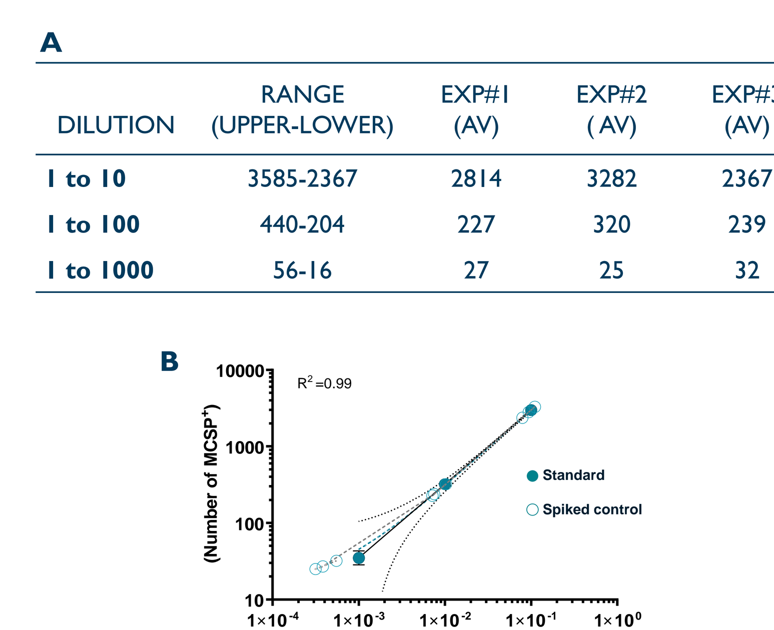


B

DILUTION	AV	SD	UPPER LIMIT (AV + 3SD)	LOWER LIMIT (AV - 3SD)
1 to 10	2976	203	3585	2367
1 to 100	322	39	440	204
1 to 1000	36	7	56	16

Figure 4. Detection of spiked controls for the determination of tumor detection accuracy. The assay was performed by spiking known amounts of tumor cells into PBMC suspensions (n=10). $MCSP^+$526 melanoma tumor cells were diluted at ratios of 1 : 10, 1 : 100, and 1 : 1,000, then mixed with PBMC and stained with anti-MCSP and anti-CD45 antibodies and live/dead dye and analyzed by flow cytometry. (**A**) Approximately 3000, 300, and 30 cells were detected in the dilution of 1:10, 1:100, and 1:1000, respectively. (**B**) An average (AV) and standard deviation (SD) of cells acquired in each condition was used to define the upper and lower reference limits.

Purity: Qualification of residual tumor detection assay using spiked controls



A

DILUTION	RANGE (UPPER-LOWER)	EXP#1 (AV)	EXP#2 (AV)	EXP#3 (AV)
1 to 10	3585-2367	2814	3282	2367
1 to 100	440-204	227	320	239
1 to 1000	56-16	27	25	32

Figure 5. Repeatability study of upper and lower limits in spiked controls. Three independent experiments were performed in triplicate to determine the repeatability of spiking assay. (**A**) The number of $MCSP^+$ detected tumor cells were consistently within the range of upper and lower reference limits. (**B**) Linear regression plot demonstrates the correlation between $MCSP^+$ cells and spiking dilutions (R^2=0.99) with the black solid line showing the best fit. The green and gray broken lines represent the 95% prediction limits in standard curve and samples (Exp#1 to 3), respectively.

Purity: Melanoma tumor cell contaminants are below the limits of assay detection in final TIL product

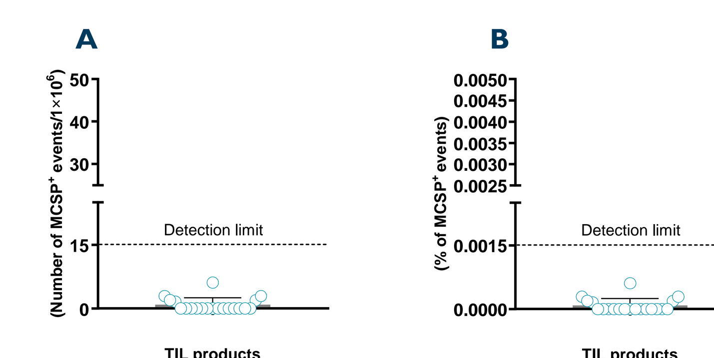


Figure 6. Detection of residual melanoma tumor in TIL products. TIL products were assessed for residual tumor contamination using the developed assay (n=15). (**A** and **B**) The median number and percentage of detectable $MCSP^+$ events was 2 and 0.0002%, respectively.

Potency: IFNγ secretion by TIL (consistently > 1000 pg/ml) demonstrates effector function of TIL product

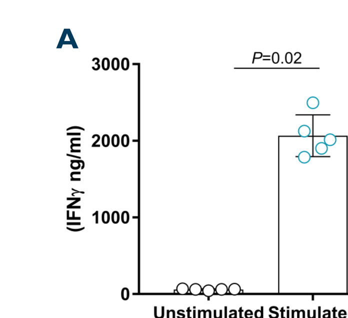


Figure 7. Potency assessment of TIL products following T-cell activation. IFNγ secretion after re-stimulation with anti-CD3/CD28/CD137 in TIL products assessed by ELISA in duplicate (n=5). (**A**) IFNγ secretion by the TIL products was significantly greater than unstimulated controls using Wilcoxon signed rank test ($P=0.02$), and consistently >1000 pg/ml. IFNγ secretion >200 pg/ml is considered to be potent. p value <0.05 is considered statistically significant.

CONCLUSIONS

- Key product parameters of identity, purity, and potency of TIL products were evaluated.
- TIL products manufactured by IOVANCE consisted of greater than 99% $CD45^+CD3^+$ T cells.
- The majority of $CD4^+$ and $CD8^+$ TIL subsets exhibited an effector-memory phenotype, associated with T-cell cytotoxic function.
- A flow cytometry-based assay to detect contaminant melanoma tumor cells in final TIL product was successfully developed and qualified.
- Applying this assay, contaminant melanoma tumor cells in final TIL product were shown to be below the limits of assay detection.
- IFNγ secretion by final TIL product following anti-CD3/CD28/CD137 re-stimulation may serve as a potency assay for commercially manufactured TIL.
- These data provide the foundation of a quality control platform that will support further development of critical quality attributes for commercial production of TIL products.

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